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APPEARANCE OF POLY(A)-RICH RNA IN GERMINATING PEA SEEDS*

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1. The affinity chromatography on poly(U)-Sepharose 4B provided evidence for
the absence of poly(A)-rich fragments in RNA from embryo axes of dry pea seeds,
and for appearance of these fragments after 2 h of swelling.

2. The percentage of the poly(A)-rich RNA fraction remained unchanged in
the initial phase of germination (up to 68 h).

3. The poly(A)-rich RNA fraction was found to promote protein synthesis in
vitro more than did the poly(A)-poor fraction.

4. Ribonuclease A and T1-resistant fragments isolated from the poly(A)-rich
RNA contain 70-80 nucleotides, 78% of which are adenine nucleotides.

5. It seems that polyadenylation of a part of mRNA preexisting in dry pea seeds
precedes the initiation of protein biosynthesis.

During the last few years evidence was provided for the occurrence of poly(A)-
rich fragments bound to RNA in several plants: soybean seedlings (Key et al.,
1972), root tips of pea seeds germinated for 72 h (Jackson & Ingle, 1973), Zea mays
roots (Van de Walle, 1973) and cotton embryos (Harris & Dure, 1974; Hammett
& Katterman, 1975).

As we have shown previously (Sielianowicz & Chmielewska, 1973), in pea
seed embryo axes the initiation of protein biosynthesis precedes RNA synthesis
de novo. Furthermore, during initiation of protein biosynthesis the blocked mRNA
pool is activated in a definite order (Sielianowicz & Chmielewska, 1974). The purpose
of the present work was to investigate the relation between polyadenylation of RNA
and activation of mRNA in germinating pea seeds.

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[59]
EXPERIMENTAL

Materials

All reagents were of analytical grade. The reagents for polyacrylamide-gel electrophoresis were purchased from Serva (Heidelberg, G.F.R.), and for the cell-free protein biosynthesis system from Calbiochem (Los Angeles, Calif., U.S.A.). Poly(U)-Sepharose 4B and Sephadex G-50 were products of Pharmacia (Uppsala, Sweden), formamide of B.D.H. Laboratory (Poole, England), ribonuclease T₁ (Aspergillus oryzae) and ribonuclease A (bovine pancreas, 5× cryst.) of P-L Biochemicals Inc. (Milwaukee, Wis., U.S.A.). The high-specific-activity¹⁴C-labelled amino acid mixture (50 μCi/ml) was purchased from the Radio-Chemical Centre (Amersham, Bucks., England).

Pea (Pisum sativum) variety "Wczesny kujawski" was used. Germination of the seeds and isolation of RNA from embryo axes was performed as described previously (Sieliwanowicz & Chmielewska, 1973).

Methods

Polyacrylamide-gel electrophoresis. This was performed in 2.2% gels according to the method described previously (Sieliwanowicz et al., 1974). The approximate molecular weight of the particular RNA fractions were calculated according to Peacock & Dingman (1968).

Poly(U)-Sepharose 4B chromatography. The column (1×5 cm) was filled with 3 ml of the gel and washed with 10 vol. of 25% formamide containing 0.7 M-NaCl and 0.05 M-Tris-HCl, pH 7.4. Thirty two A₂₆₀ₐₚ units of RNA was then applied onto the column and the non-retained fraction was eluted with 3 vol. of the same buffer. Fractions retarded on the column were eluted with 2 vol. of 90% formamide in 0.01 M-Tris-HCl, pH 7.4. Each fraction was dialysed against 1000 vol. of distilled water and lyophilized.

Incorporation of ¹⁴C-labelled amino acids in vitro. Incorporation of the ¹⁴C label was assayed in the cell free system described previously (Jachymczyk et al., 1974).

Digestion of RNA. Non-fractionated RNA of embryo axes and the poly(A)-rich RNA fraction were digested with ribonuclease A (2 μg) and T₁ (4 units) per 3-5 mg of RNA in a total volume of 0.5 ml, according to Green & Fausto (1974).

Sephadex G-50 chromatography. This was performed on a 40×1.2 cm column, equilibrated with 0.1 M-NaCl in 0.01 M-sodium citrate. Fractions of 2 ml were eluted with the same buffer, and RNA content was determined spectrophotometrically at 260 nm (Zeiss VSO-2P spectrophotometer).

Determination of nucleotide composition. The nucleotide composition of non-fractionated RNA, the fractions separated by poly(U)-Sepharose 4B chromatography, and the poly(A)-rich RNA digestion products was quantitatively determined according to the method of Cohn & Volkin (1953).
RESULTS AND DISCUSSION

Poly(A)-rich RNA, retardable on poly(U)-Sepharose 4B, was absent in the RNA isolated from dry pea seeds. The content of AMP in the retarded material (about 1% of the applied amount) was not higher than in the non-fractionated RNA. However, swelling of the seeds for 2 hours was found to be sufficient for generation of the poly(A)-rich RNA fraction (Table 1). This fraction, retained on the column, amounted to 7-9% of the non-fractionated RNA, irrespective of the time of germination (22, 44 or 68 h).

Table 1

<table>
<thead>
<tr>
<th>Germination time (h)</th>
<th>RNA, non-fractionated (units)</th>
<th>RNA non-retained</th>
<th>retained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units</td>
<td>%</td>
<td>units</td>
</tr>
<tr>
<td>0</td>
<td>31.9</td>
<td>101.5</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>32.8</td>
<td>92.4</td>
<td>2.90</td>
</tr>
<tr>
<td>22</td>
<td>33.5</td>
<td>94.6</td>
<td>3.12</td>
</tr>
<tr>
<td>44</td>
<td>32.6</td>
<td>94.8</td>
<td>2.28</td>
</tr>
<tr>
<td>68</td>
<td>33.1</td>
<td>95.5</td>
<td>2.41</td>
</tr>
</tbody>
</table>

The comparative analysis of nucleotide composition of RNA and its fractions showed that AMP content in the RNA fraction retained on poly(U)-Sepharose 4B was raised to 33% at the expense of UMP (Table 2). It is of considerable interest that the poly(A)-rich fraction was not increased on prolonged germination (up to 68 h).

Table 2

<table>
<thead>
<tr>
<th>RNA</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
</tr>
<tr>
<td>Non-fractionated</td>
<td>20</td>
</tr>
<tr>
<td>Fractionated</td>
<td></td>
</tr>
<tr>
<td>Non-retained</td>
<td>18</td>
</tr>
<tr>
<td>Retained</td>
<td>33</td>
</tr>
</tbody>
</table>

Nucleotide composition of RNA from pea seeds

Non-fractionated RNA from pea seeds swollen for 2 h and the fractions separated on poly (U)-Sepharose 4B were analysed as described under Methods.
As it could be expected, molecular weight of the poly(A)-rich RNA corresponded to that for mRNA (Fig. 1). The RNA fraction of lower adenine content, not retained on the column, consisted mainly of rRNA and tRNA.

Fig. 1. Electrophoretic pattern of RNA isolated from pea seeds germinated for 22 h. The non-fractionated RNA (a) and the RNA fractions: non-retained (b) and retained (c) on poly(U)-Sepharose 4B were analysed by polyacrylamide-gel electrophoresis. For details see Methods.

Fig. 2. Sephadex G-50 elution profiles of RNA digested by the mixture of ribonucleases A (bovine pancreas, 2 µg) and T₁ (A. oryzae, 4 units). ———, Non-fractionated RNA; ————, poly(A)-rich RNA digestion products. All the digestion products were insoluble in 70% ethanol and showed up to 25% of hyperchromicity.

To confirm the presence of mRNA in the poly(A)-rich RNA fraction, the template activity of non-fractionated RNA and its fractions separated on poly(U)-Sepharose 4B, were compared. It was found (Table 3) that the addition of the poly(A)-rich fraction stimulated about threefold incorporation of ¹⁴C-labelled amino acids. Stimulation by the non-fractionated RNA and the RNA fraction of lesser adenine content was considerably lower.

To determine the composition and molecular weight of the poly(A)-rich fragments in the RNA fraction retained on poly(U)-Sepharose 4B, this fraction was degraded by ribonucleases A and T₁. Figure 2 presents the Sephadex G-50 elution profiles of the digestion products of the non-fractionated RNA and the poly(A)-rich RNA fraction. The fraction designated A was eluted at the same volume as the ribonucleases used. Fraction B contained polynucleotides, and fraction C oligonucleotides of
low molecular weight. Significant differences were observed in quantitative content of fraction B in the digestion products of non-fractionated RNA (10%) and the poly(A)-rich RNA (20% of the material applied on the Sephadex G-50 column).

**Table 3**

*The template activity of RNA from pea seeds germinated for 22 hours*

The complete system for protein biosynthesis contained in a volume of 1 ml: 0.8 mg protein of "pH 5 enzymes"; 1.0 mg of ribosomes; RNA or RNA fractions separated on poly(U)-Sepharose 4B, and 14C-labelled amino acids (0.5 μCi). The incubation was carried out at 37°C for 30 min.

<table>
<thead>
<tr>
<th>RNA</th>
<th>Incorporation of 14C-labelled amino acids (c.p.m./mg of ribosomes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2600</td>
</tr>
<tr>
<td>Non-fractionated (200 μg)</td>
<td>4900</td>
</tr>
<tr>
<td>Poly(A)-poor (200 μg)</td>
<td>4200</td>
</tr>
<tr>
<td>Poly(A)-rich (100 μg)</td>
<td>8200</td>
</tr>
</tbody>
</table>

Molecular weight of fraction B calculated from the electrophoretic mobility in 2.2% polyacrylamide gel, was 25,000 - 30,000 daltons which corresponds to chain length of about 70 - 80 nucleotides. A similar length of the poly(A)-rich RNA fraction was found in other plants (Jackson & Ingle, 1973).

**Table 4**

*Nucleotide composition of ribonuclease-resistant fragments of the poly(A)-rich RNA fraction*

RNA was isolated from embryo axes of pea seeds germinated for 22 h. The poly(A)-rich fraction separated on poly(U)-Sepharose 4B was digested with ribonucleases A and T1, and the digestion products obtained were fractionated on Sephadex G-50 column (cf Fig. 2).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mole %</th>
<th>AMP</th>
<th>CMP</th>
<th>UMP</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>78</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>34</td>
<td>16</td>
<td>23</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

The nucleotide composition of fraction B from the poly(A)-rich RNA fraction (Table 4) indicates that this fraction contained 78% of adenine nucleotides, similarly as the corresponding fractions from Zea mays root RNA (Van de Walle, 1973), but at variance with that of cotton embryos (Hammett & Katterman, 1975) and callus tissue derived from rice roots (Manecham et al., 1973).

The data presented above give evidence for the absence of poly(A)-rich RNA fraction in the embryo axes of dry pea seeds; in this respect these seeds differ essentially from cotton embryos (Hammett & Katterman, 1975). It seems very interesting
that the initiation of protein synthesis in embryo axes of pea seeds swollen for 2 hours
(Sieliwanowicz & Chmielewska, 1973) is closely correlated with the appearance of
poly(A)-rich RNA.

REFERENCES


POJAWIENIE SIĘ BOGATEJ WE FRAGMENTY POLIADENYLOWE FRAKCJI RNA
W CZASIE KIEŁKOWANIA NASION GROCHU

Streszczenie

1. Chromatografia powinowactwa na poly(U)-Sepharose 4B wykazała brak bogatych w poli(A)
fragmentów RNA w osiach zarodkowych spoczynkowych nasion grochu i ich pojawianie się już
po 2 godz. płodnienia.
2. Procentowa zawartość bogatej w poli(A) frakcji RNA nie ulega zmianie w początkowym
okresie kielkowania (do 68 godz.).
3. Bogate w poli(A) frakcja RNA stymuluje biosyntezę białka in vitro intensywnie niż RNA
pozbawiony tej frakcji.
4. Oporne na działanie rybonukleaz A i T1, fragmenty RNA izolowane z frakcji bogatej w poli(A)
zawierają 70 - 80 nukleotydów, wśród których 78% stanowią nukleotydy adenylowe.
5. Sugieruje się, że poliadenylacja części mRNA istniejącego w spoczynkowych nasionach
grochu poprzedza uruchomienie procesu biosyntezy białka.

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